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Induction of Interferon Synthesis in Mice by Fractions from *Nocardia*

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Three fractions of *Nocardia*, *Nocardia* water-soluble mitogen (NWSM), *Nocardia* water-soluble mitogen pellet (NWSMP), and the cell wall peptidoglycan, which are mitogenic for B lymphocytes, were able to induce circulating interferon in mice, NWSMP and NWSM being the most active. The peak of interferon appeared about 2 h after the injection. The interferon induced by NWSMP and NWSM was acid stable and antigenically related to viral interferon, as shown by neutralization with antibodies directed against Newcastle disease virus-induced interferon.

The preparation of adjuvant and mitogenic fractions from *Nocardia* has been described previously (2, 3, 6-8). Two of these fractions, *Nocardia* water-soluble mitogen (NWSM) and *Nocardia* water-soluble mitogen pellet (NWSMP), are derived from the products solubilized by the action of lysozyme on delipidated cells.

NWSM and NWSMP induce a strong proliferation of spleen lymphocytes in AKR and nude mice (2). NWSM is also a B mitogen in the rabbit, as proved by its mitogenic activity on spleen cells deprived of T cells by treatment with anti-rabbit thymus lymphocyte antigen serum and complement (1), and in humans, as proved by its mitogenicity on B lymphocytes from blood, spleen, and tonsils (5). NWSM induces a polyclonal activation in vitro in all species mentioned (1, 2, 5). The peptidoglycan and soluble peptidoglycan fractions from *N. opaca* and *N. rubra* stimulate the spleen lymphocytes of rabbits and AKR or nude mice (8).

As some B mitogens of bacterial origin, such as the lipopolysaccharide (LPS) of *Escherichia coli*, are able to induce interferon after intravenous (i.v.) injection in mice (15), we have investigated whether the *Nocardia* mitogens are also interferon inducers. It must be noted, however, that whereas LPS has been shown to induce blast transformation and polyclonal activation both in vitro and in vivo, NWSM produces these responses in vitro only (11).

MATERIALS AND METHODS

Preparation and mitogenic activity of *Nocardia* fractions. NWSM and NWSMP were obtained as previously described (6). NWSM is the first fraction (fraction A) obtained by Sephadex G75 filtration of the products solubilized by egg white lysozyme acting on delipidated cells.

NWSMP is prepared by suspending lyophilized NWSM in glacial acetic acid. After centrifugation for 1 h at 48,000 × *g* at 4°C, the pellet is washed twice with glacial acetic acid by repeated suspensions and centrifugations. The final pellet is lyophilized and is called NWSMP; it is soluble at a slightly alkaline pH. It is devoid of the cell wall fragments contained in NWSM (6, 7).

The peptidoglycan of *N. rubra* was obtained from cell walls prepared by pressure disruption of fresh cells followed by differential centrifugations. To obtain the peptidoglycan fraction, the cell walls are treated with proteolytic enzymes, delipidated, submitted to a mild acid hydrolysis (HCl, 0.1 N; 60°C; 12 h), and redelipidated (7, 8).

As far as mitogenic activity is concerned, in comparative experiments, NWSMP was found to be approximatively three times more active and NWSM two times more active than peptidoglycan (6-8).

Interferon induction. Swiss female mice (Elevage Janvier, Le Genest F53680), 8 to 12 weeks old, were inoculated i.v. with the *Nocardia* products dissolved or suspended in a final volume of 100 μl of saline. Mice were bled by decapitation at the indicated time, and the sera were stored at −20°C until used. In most experiments, the pooled sera of 10 mice were used.

Interferon titrations. Titrations were performed by the cytopathogenic inhibition test on L-cell cultures in microplates using vesicular stomatitis virus as challenge (10). Titers were expressed in international reference units.

Acid stability. Interferon samples diluted to 200 U/ml were dialyzed for 24 h at 4°C against saline, pH 2, followed by a dialysis against phosphate buffer, pH 7.4. Controls were dialyzed only against phosphate buffer for the same time. Interferon titers of treated and control samples were then measured simultaneously.

Antiserum. Rabbit anti-mouse L-cell interferon

serum was prepared in the Laboratoire de Biochimie Virale (Institut du Radium, Paris) by inoculating repeatedly semipurified mouse L-cell interferon induced with Newcastle disease virus (NDV) (specific activity, 10^7 U/mg of protein). The first two inoculations were 2×10^6 U of interferon followed, for 6 months, by inoculation of 1×10^5 U of interferon each 4 weeks.

In vitro neutralization test. Interferon neutralization titers were determined essentially as described by Ogburn et al. (12). Briefly, 10 U of each interferon preparation was mixed with an equal volume of a serial twofold dilution of anti-interferon serum and incubated at 37°C for 60 min. An aliquot of each mixture was then placed, in duplicate, in wells of microtray cultures. The reciprocal of the dilution of antiserum that permits the development of 50% of the cytopathogenic effect was considered the titer of antiserum that neutralizes 10 U of the preparation of interferon tested.

RESULTS

Time course of circulating interferon induction. Kinetics of appearance of interferon in the blood depends on the inducer; e.g., the maximum level of interferon is attained 4 to 7 h after i.v. injection of large amounts of viruses (like NDV) into mice (9), whereas a nonviral inducer, like LPS, leads to the maximum interferon response 2 to 3 h after injection by the same route (15). To establish the kinetics of induction of circulating interferon by *Nocardia* fractions in mice, the interferon level induced by the i.v. injection of different doses of NWSM was measured in the serum at different times during 48 h. The peak of interferon activity appeared 2 h after injection of NWSM, whatever the dose used between 50 and 400 μg (Fig. 1). The amount of interferon in the serum is dose dependent, the highest titer being obtained with the highest dose of the inducer. The circulating interferon disappeared very rapidly; less than 10% of the amount of interferon present at 2 h was detected 24 h after injection.

Table 1 gives the titers obtained with various doses of NWSM, NWSMP, and peptidoglycan tested in the same experiment. None of the doses used had toxic effects. All of the samples showed interferon-inducing properties, although with different efficacy. In three independent experiments, the highest titers were consistently obtained with 200 μg of NWSMP per mouse, whereas as much as 400 μg of NWSM induced a lower amount of interferon. Peptidoglycan also induced circulating interferon with similar kinetics, but titers were significantly lower. The same order of relative activities of these three products was also observed when their mitogenic activity was compared (2, 3, 6–8).

TABLE 1. *Titers of circulating interferon in mice inoculated i.v. with NWSM, NWSMP, and peptidoglycan*

Interferon inducer	Dose (μg/mouse)	Titer[a] (U/ml of serum)				
		1 h[b]	2 h	3 h	6 h	24 h
NWSM	200	30	210	180	45	25
	400	35	320	120	27	27
NWSMP	200	27	480	200	27	27
	400	20	480	200	20	10
Peptidoglycan	200	<9	80	<9	<9	<9
	400	9	15	70	20	<6

[a] Before administration of *Nocardia* fractions, the interferon titer measured in the serum was 9 U/ml.
[b] Bleeding time.

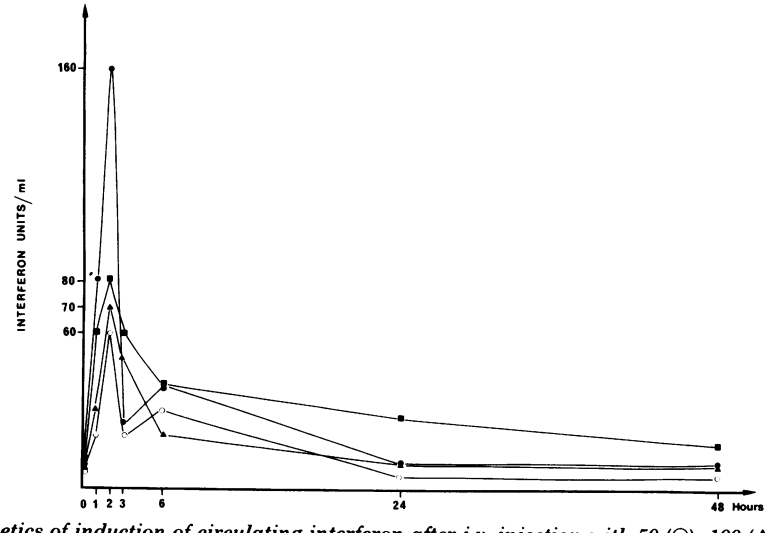

FIG. 1. *Kinetics of induction of circulating interferon after i.v. injection with 50 (○), 100 (▲), 200 (■), and 400 (●) μg of NWSM.*

Neutralization of NWSM and NWSMP induced interferon with antiviral interferon antibodies and stability at acid pH. It has been recently reported (13) that interferon induced by nonviral inducers can be classified in types B and T interferons according to the specificity of the inducer towards lymphocytes. The most striking differences between both types are linked to their antigenic properties and stability at an acid pH. The interferon induced by LPS, which is a B mitogen, is similar to the viral-induced interferon: it is neutralized by antiviral interferon antibodies and is stable at pH 2 (9, 14), whereas the interferon induced by T mitogens, such as phytohaemagglutinin, is not neutralized by the antiviral interferon serum and is unstable at pH 2 (13).

As NWSM and NWSMP are known to be active on B cells in vitro, we compared the antigenic properties and acid stability of the interferons they induce to a virus-induced interferon, the NDV L-cell interferon.

Both interferons are stable at pH 2 and are neutralized by the anti-NDV interferon serum with a neutralization titer similar to that presented by the NDV L-cell interferon (Table 2).

DISCUSSION

Fractions of *Nocardia* (NWSM, NWSMP, and peptidoglycan) showing different degrees of mitogenic activity were tested as interferon inducers. All three fractions were able to induce the synthesis of interferon when inoculated i.v. into mice, although, on a weight basis, NWSMP gave the highest titers, peptidoglycan being the less active. These results correlate with their respective mitogenic activity.

NWSMP differs from NWSM, from which it is obtained, by the absence of cell wall components; therefore, the active constituent(s) for the interferon induction in NWSM is not derived from the cell wall, but very probably from the cytoplasmic membrane (6–8). Further progress in the purification of *Nocardia* fractions will allow us to determine whether the same molecule(s) is responsible for both mitogenicity in vitro and interferon induction in vivo.

Like other B mitogens (e.g., LPS), NWSM and NWSMP induce B-type interferons (equivalent of type I interferon of Youngner [14]) related to viral-induced interferon, as evaluated by their antigenic properties and pH stability. It must be noted, however, that, in contrast to LPS, NWSM administered to mice in vivo failed to induce proliferation of lymphocytes and polyclonal activation (11). Nevertheless, like LPS, NWSM activates murine macrophages both in vivo and in vitro (4). These results suggest at least two possible hypotheses. (i) The target cell for interferon induction by LPS and NWSM is not necessarily the B lymphocyte only, but could require the participation of another cell, such as the macrophage; and (ii) the level of stimulation required for interferon induction is different from the level required for blast transformation and immunoglobulin synthesis.

TABLE 2. *Acid stability and in vitro neutralization of NWSM-, NWSMP-, and NDV-induced interferons by anti-NDV interferon serum*[a]

Interferon inducer	Antiserum titer against 10 interferon units	Residual activity after pH 2 treatment (%)
NDV	15,000	100
NWSM	12,000	80
NWSMP	15,000	100

[a] As described in the text.

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